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The role of CRF receptor subtypes in stress-induced behavioural responses

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Abstract

The actions of corticotropin-releasing factor (CRF) and CRF-related peptides in the brain and periphery are mediated through multiple receptors. Two CRF receptor subtypes that differ markedly in their pharmacological profiles and anatomical distribution have been identified and characterized. Important advances have been made in understanding CRF and its actions through the development of specific CRF receptor antagonists, application of antisense oligonucleotides, and the production of transgenic mice lacking functional CRF_1 receptors. This chapter describes recent findings with respect to CRF-related peptides and CRF receptors and their role in stress-induced behaviours.

Keywords: CRF (corticotropin-releasing factor); Urocortin; (Antagonist); CRF receptor; Antisense; Stress; Behaviour

1. Introduction

Stress, a common factor in everyday life, is associated with many problems, including exacerbation of psychiatric diseases. It was David de Wied who first recognized that components of the hormonal cascade known as the hypothalamo-pituitary-adrenocortical axis and thought to be a critical physiological response in stress, had biological activities other than their endocrine ones. His classic work showed that both adrenocorticotrophic hormone (ACTH) and vasopressin had behavioural activity. Nevertheless, the effects of ACTH and vasopressin were comparatively subtle, and not all researchers were convinced that these behavioural activities were physiologically significant. In 1981, Vale et al. (1981) completed the sequencing and synthesis of corticotropin-releasing factor (CRF). With the availability of the synthetic peptide, it rapidly became apparent that CRF had potent behavioural activity (Koob and Bloom, 1985) which was more clearly related to the effects of stress than those associated with ACTH or vasopressin. Thus, it is apt to honour David with this update on the behavioural activities of CRF and the mechanisms involved.

CRF is a mediator of endocrine, autonomic and immune responses in stress (De Souza, 1995; Dunn and Berridge, 1990; Owens and Nemeroff, 1991; Vale et al., 1981) and it has been suggested that CRF may also coordinate autonomic and behavioural responses in stress, including anxiety-like behaviours, food intake, arousal, learning and memory (De Souza, 1995; Dunn and Berridge, 1990; Heinrichs and Richard, 1999; Koob and Bloom, 1985; Liang and Lee, 1988; Sutton et al., 1982). In the past 5 years, there have been important advances in understanding the physiology of the CRF system and its response to stress. Receptors mediating the action of CRF have been identified and cloned, and their distribution in the brain and peripheral organs has been characterized (Chalmers et al., 1995; Lovenberg et al., 1995a; Sánchez et al., 1999). Also, urocortin, a newly discovered peptide of the CRF family, has been identified in the brain and peripheral organs of mammals (Donaldson et al., 1996; Kageyama et al., 1999; Vaughan et al., 1995).

The role of CRF receptor subtypes in emotional processes and its relation to depression and anxiety behaviours has been recently reviewed (Holsboer, 1999; Steckler and Holsboer, 1999). The purpose of this article is to highlight recent findings on the role of different compo-

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nents of the CRF system in the behavioural responses to stress, and in particular, the roles of the distinct CRF receptor subtypes.

2. Anatomy of the CRF system

2.1. Peptides: CRF and urocortin

The anatomy of the CRF system has been described in detail (Sawchenko and Swanson, 1985, 1990; Swanson et al., 1983) and is summarized in Fig. 1. High densities of CRF-immunoreactive neurons have been found in the paraventricular nucleus of the hypothalamus, the major locus of CRF-containing cell bodies. Hypothalamic CRF is released into the portal vessels and is carried in the blood to the anterior pituitary from which it activates the release of ACTH (Vale et al., 1981) triggering the activation of the hypothalamo-pituitary-adrenocortical axis. Extrahypothalamic CRF-containing neurons have been found in the central nucleus of the amygdala, the bed nucleus of the stria terminalis, the lateral hypothalamic area, the parabrachial nucleus, the hippocampus, the nucleus accumbens, and the cerebellum (Merchenthaler et al., 1982, 1983, 1984; Sakanaka et al., 1987; Sawchenko and Swanson, 1985; Swanson et al., 1983).

Vaughan et al. (1995) characterized another member of the CRF family in mammals and named it urocortin, because the peptide is related to urotensin (63% sequence identity) and CRF (45% sequence identity) (Table 1). The major sites of urocortin mRNA expression in the rat brain are the Edinger–Westphal nucleus, with lesser amounts in the lateral superior olive, the hippocampus, the basal ganglia, the medial septum, the paraventricular nucleus of the

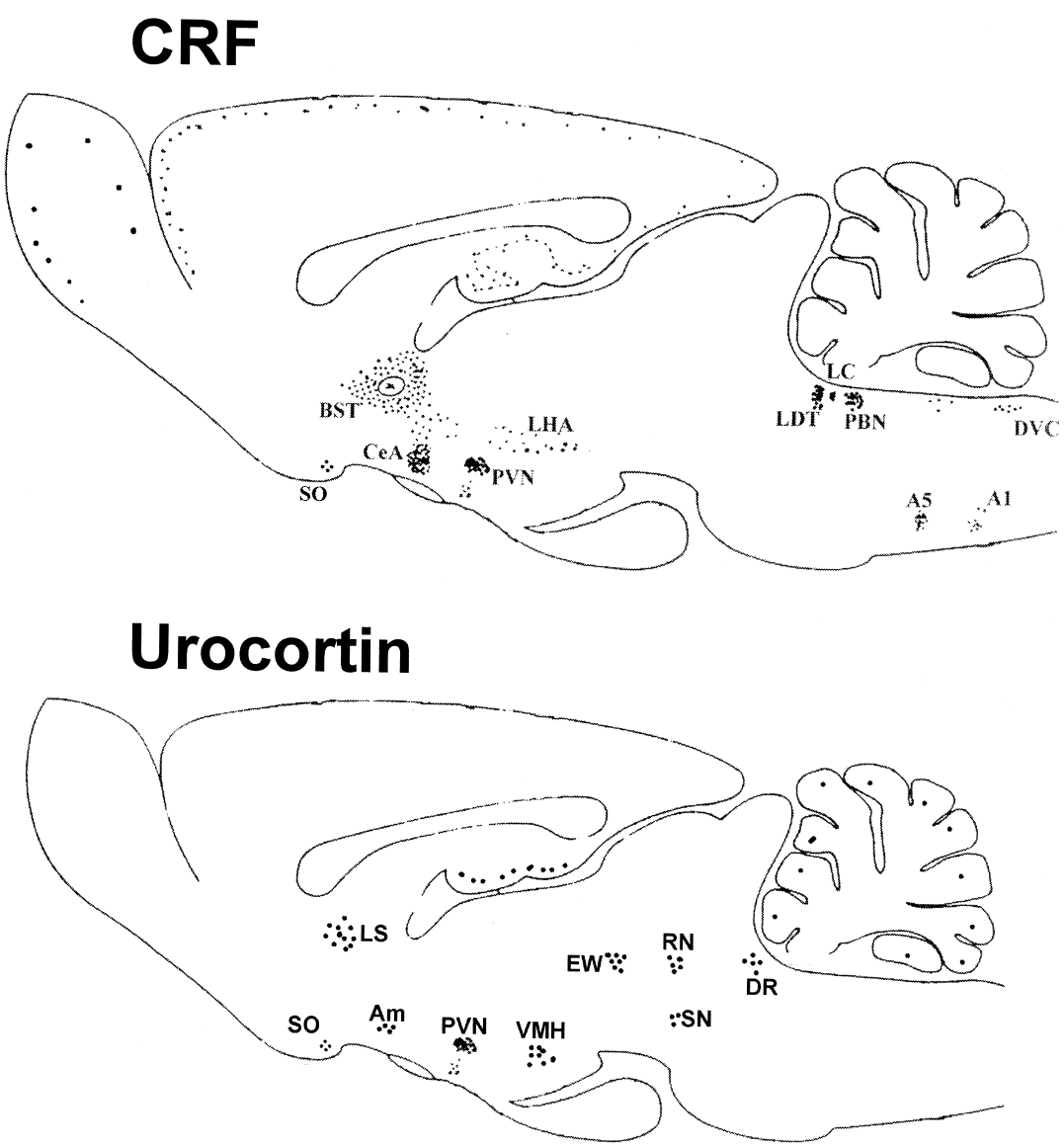

Fig. 1. Comparison of the brain distribution of CRF- and urocortin-immunoreactive cells in the rodent brain. Data taken from (Behan et al., 1996; Kozicz et al., 1998; Yamamoto et al., 1998; Yanaihara et al., 1997). All abbreviations are taken from Paxinos and Watson's (1982) Brain atlas.

Table 1
Homology of CRF peptide family
Amino acid sequences of the CRF-like peptides. Identical amino acids are set in boldface type.

Human/rat CRF	S E E **P P I S** L **D L T F HL L R** E V L **E** M A R A E Q L A Q Q **A** H S **N R** K L M E I I
Rat urocortin	D D **P P L S** I **D L T F HL L R** T L L **E** L A R T Q S Q R E R **A** E Q **N R** I I F D S V
Urotensin I	N D D **P P I S** I **D L T F HL L R** N M I **E** M A R I E N E R E Q **A** G L **N R** K Y L D E V
Sauvagine	Z G **P P I S** I **D L** S L E **L L R** K M I **E** I E K Q E K E K Q Q **A** A N **N R** L L L D T I

hypothalamus, and the lateral hypothalamus (Vaughan et al., 1995; Wong et al., 1996). Immunohistochemical studies using an antibody specific for urocortin, found immunoreactivity in the supraoptic, ventromedial hypothalamic nuclei, the paraventricular nucleus of the hypothalamus, the dorsal tegmental nucleus, the dorsal raphe nuclei and the substantia nigra. The most abundant urocortin-immunoreactive perikarya were found in the Edinger–Westphal nucleus (Kozicz et al., 1998; Yanaihara et al., 1997). In another study, only a few urocortin-immunoreactive neurons were found in the hypothalamus, but a dense fiber network was found in the lateral septal area (Morin et al., 1999). However, no fibers were observed in the medial eminence or the pituitary. A summary of the distribution of urocortin-containing cell groups is illustrated in Fig. 1.

The limited overlap between the distributions of CRF and urocortin in the rat brain suggests that these two peptides have distinct physiological roles, but the role of

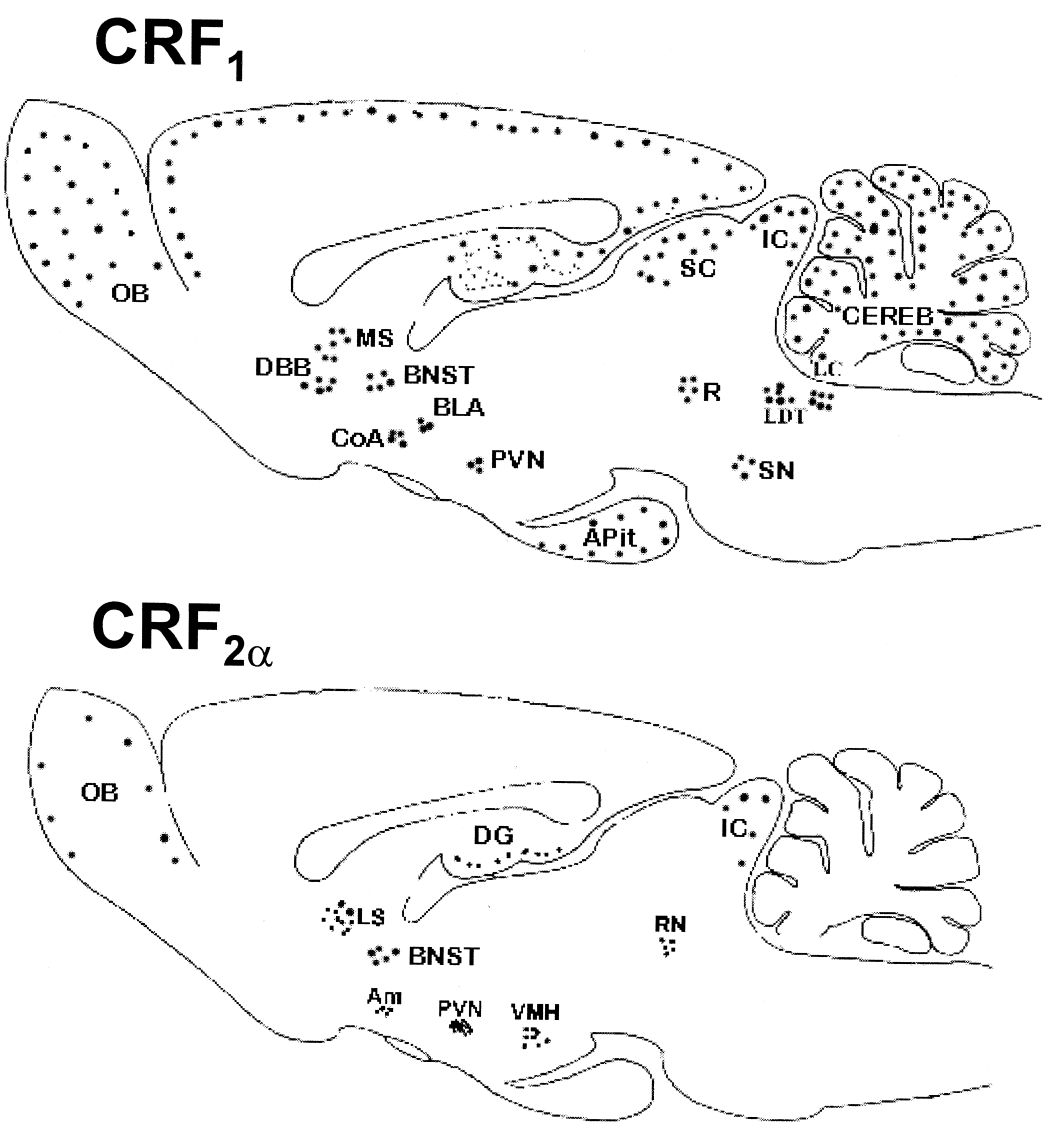

Fig. 2. Comparison of the distribution of CRF_1 and CRF_2 binding sites in the brain. The localization of CRF_1 receptors in the LC is based on primate data (Sánchez et al., 1999). Modified from Steckler and Holsboer (1999). All abbreviations are taken from Paxinos and Watson (1982).

urocortin has not yet been elucidated. Studies using antisera to urocortin have indicated that it is not a significant mediator of ACTH release in response to foot-shock or adrenalectomy (Masuzawa et al., 1999; Turnbull et al., 1999). In a recent study of CRF knockout mice, normal and CRF-deficient mice had very similar distributions of urocortin mRNA, as determined by in situ hybridization (Weninger et al., 1999). There was no ectopic urocortin mRNA expression in CRF-deficient mice in areas that normally express CRF. However, urocortin mRNA in the Edinger–Westphal nucleus was increased approximately three-fold after 3 h restraint (Weninger et al., 2000). Because the Edinger–Westphal nucleus is not known to project to any brain regions believed to play a role in anxiety-related behaviour, the existence of another CRF-like molecule (other than CRF and urocortin), has been proposed (Weninger et al., 1999, 2000).

2.2. CRF receptor subtypes and their role in behaviour

The structure of the cDNA encoding the human pituitary CRF receptor has been characterized by Chen et al. (1993). It encodes a 415-amino acid protein (designated the CRF_1 receptor). CRF_1 receptors have also been identified in the rat brain (Chang et al., 1993; Perrin et al., 1993). Lovenberg et al. (1995a) identified a rat brain cDNA clone that encodes a second CRF receptor. The CRF_2 receptor gene encodes a protein of 411 amino acids and has 70% identity with the rat CRF_1 receptor over the entire coding region. An additional splice variant of the CRF_2 receptor with a different N-terminal domain, encoding a 431-amino acid protein, has been identified and designated the $CRF_{2\beta}$ receptor. CRF_1 and $CRF_{2\alpha}$ receptors clearly have different tissue distributions (Lovenberg et al., 1995b). Prominent expression of the $CRF_{2\alpha}$ receptor was found in the lateral septum, the ventromedial nucleus of the hypothalamus, and several amygdaloid nuclei (Lovenberg et al., 1995b). $CRF_{2\alpha}$ receptor mRNA was not detected in the neocortex and cerebral cortex, in contrast to the high levels of CRF_1 receptor expression in these regions (Lovenberg et al., 1995b). Similarly, $CRF_{2\alpha}$ receptor expression was almost undetected in the pituitary lobes, where CRF_1 receptor expression is high (Chalmers et al., 1995; Lovenberg et al., 1995b). Receptor binding studies have also been performed to determine the distribution of binding sites for CRF_1 and CRF_2 receptors (Gottowik et al., 1997; Grigoriadis et al., 1996; Primus et al., 1997; Rominger et al., 1998; Sánchez et al., 1999). These data are summarized in Fig. 2.

CRF_1 and $CRF_{2\alpha}$ receptors differ in their regulation in response to a variety of stressors. Various stressors have been shown to upregulate CRF_1 mRNA in the paraventricular nucleus of the hypothalamus (Lacroix and Rivest, 1996; Lee and Rivier, 1997; Mansi et al., 1996), suggesting that this receptor subtype might primarily mediate the effect of stress on the hypothalamo-pituitary-adrenocortical axis. The role of $CRF_{2\alpha}$ receptors has been attributed largely to behaviours such as maternal deprivation and feeding, since it was shown that $CRF_{2\alpha}$ mRNA is decreased in response to food deprivation and maternal deprivation in rats (Eghbal-Ahmadi et al., 1997, 1998; Makino et al., 1998; Timofeeva and Richard, 1997).

The role of CRF receptors in stress-related behaviours has been assessed using various approaches: by blocking the receptors using selective and nonselective receptor antagonists; by downregulating expression of the receptor protein using antisense oligonucleotides, and by producing mice lacking specific receptors.

3. CRF receptor antagonists

The first CRF receptor antagonist described was α-helical CRF(9–41) (αhCRF(9–41)) (Rivier et al., 1984). At high doses it prevented the CRF- and stress-induced ACTH secretion from the pituitary and has been used in many behavioural experiments. These studies provided support for a role of CRF in behavioural and neuroendocrine responses to stress reviewed earlier (Britton et al., 1986; Heinrichs and Koob, 1992; Heinrichs et al., 1992; Koob et al., 1993; Dunn and Berridge, 1990; Owens and Nemeroff, 1991). Other peptide CRF receptor antagonists became available later, such as [D-Phe^{12},Nle^{21,38},Cα MeLeu^{37}] hCRF(12–41) (abbreviated as D-Phe CRF(12–41)), a more potent antagonist of CRF receptors than ahCRF. Astressin, [cyclo(30–33){D-Phe^{12},Nle^{21,38},Glu^{30},Lys^{33}}hCRF(12–41)], is significantly more potent at inhibiting ACTH secretion when administered peripherally than any of the other analogs (Gulyas et al., 1995). However, αhCRF(9–41), D-Phe CRF(12–41) and astressin bind to both subtypes of CRF receptors and thus do not permit distinctions between the roles of the specific CRF receptor subtypes (Table 2).

Several pharmaceutical companies performed a high-speed screening of chemical libraries for CRF receptor binding, yielding a group of compounds with specific affinity for CRF_1 receptor subtypes (Table 2). CP-154,526 (butyl-ethyl-(2,5-dimethyl-7-[2,4,6-trimethylphenyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amine), was produced by Pfizer (Schulz et al., 1996). Administered peripherally, this compound penetrates the CNS and blocks the effect of CRF on the acoustic startle reflex, used as an indicator of fear and anxiety (Schulz et al., 1996). Interestingly, CP-154,526 was able to block the CRF-induced increase in the firing rate of the locus coeruleus (LC) neurons (Curtis et al., 1997), suggesting that activation of the LC by CRF might be mediated by CRF_1 receptors located in or close to the LC. CP-154,526 has been demonstrated to have anxiolytic properties in studies using animal behavioural models involving stress (Griebel et al., 1998; Lundkvist et al., 1996). CP-154,526 has also been reported to have antidepressant effects in rats exposed to inescapable foot-shock (Mansbach et al., 1997).

Table 2
Pharmacological binding characteristics of CRF receptor antagonists

Compound	K_i (nM), CRF_1 receptors	K_i (nM), CRF_2 receptors	Reference
α hCRF(9–41)	40	96.2	(Behan et al., 1996)
D-Phe-CRF(12–41)	30	24	(Behan et al., 1996)
Astressin	2.0		(Gulyas et al., 1995)
CP154,526	2.7	> 10	(Schulz et al., 1996)
CRA1000	15.7[a]	> 100,000[a]	(Okuyama et al., 1999)
NBI27914	1.7		(Chen et al., 1996)
Antalarmin	1.9		(Webster et al., 1996)
Antisauvagine-30	1.4[b]	153.6[c]	(Rühmann et al., 1998)

[a] IC50 for ^{125}I-ovine CRF binding to pituitary and heart membranes.
[b] K_d displacing [^{125}I-Tyr^0]Svg.
[c] K_d displacing [^{125}I-Tyr^0]oCRF.

NBI27914 (2-methyl-4(*N*-propyl-*N*-cyclopropanemethylamino)-5-chloro-6-(2,4,6-trichloroanilino)pyrimidine), another CRF receptor antagonist, chemically related to CP-154,526, was synthesized by Neurocrine Biosciences (Chen et al., 1996) and later developed as a research tool as antalarmin (*N*-butyl-*N*-ethyl-(2,5,6-trimethyl)-7-[2,4,6-trimethylphenyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl-amine) (Webster et al., 1996). It has been reported that it binds specifically to CRF_1 receptors and that it can suppress CRF-induced ACTH release in vitro (Webster et al., 1996). This compound shows anxiolytic properties in the elevated plus-maze and stress- and CRF-induced defensive withdrawal behaviour (Smagin et al., 1998b). Administration of antalarmin impaired both the induction and expression of conditioned fear. In addition, antalarmin blocked the enhancement of fear conditioning produced by prior exposure to inescapable shock. Despite its behavioural effects, antalarmin had no effect on the inescapable shock-induced elevations of ACTH and corticosterone (Deak et al., 1999), although it attenuated the ACTH response to a brief period of restraint (Webster et al., 1996).

Recently, CRA1000 (2-[*N*-(2-methylthio-4-isopropylphenyl)-*N*-ethylamino]-4-[4-(3-fluorophenyl)-1,2,3,6-tetrahydropyridin-1-yl)-6-methylpyridimidine) and CRA1001 (2-[*N*-(2-bromo-4-isopropylphenyl)-*N*-ethylamino]-4-[4-(3-fluorophenyl)-1,2,3,6-tetrahydropyridin-1-yl)-6-methylpyrimidine), new CRF_1 selective receptor antagonists have been described (Okuyama et al., 1999). They bind to CRF_1 receptors with high specificity. In studies in mice, CRA1000 and CRA1001, administered orally, reversed the swim stress-induced reduction of the time spent in the light area in the light/dark box. However, they had no effect on the time spent in the light area in the same task. Orally administered to rats, these compounds reversed the CRF-induced reduction of time spent in open arms in elevated plus-maze (Okuyama et al., 1999).

A selective antagonist, [D-Phe^{11},His^{12}]Svg$_{(11-40)}$, directed against $CRF_{2\beta}$ receptors has recently been developed. This antagonist has been called antisauvagine-30 (Rühmann et al., 1998). It binds to $CRF_{2\beta}$ receptors with a higher affinity (109:1) than to CRF_1 receptors (Rühmann et al., 1998). In context- and tone-dependent fear conditioning of mice, the enhanced retention caused by injections of CRF into the dorsal hippocampus before training was blocked by astressin, but not by antisauvagine-30 (Radulovic et al., 1999). In contrast, the impairment of fear conditioning observed after intraseptal application of CRF was mediated by CRF_2 receptors, as indicated by the ability of both astressin and antisauvagine-30 to block this effect. Using antisauvagine-30, originally developed as a selective CRF2β receptor antagonist, indicates that CRF_2 α and β splice variants share similar ligand-binding properties (Donaldson et al., 1996).

3.1. Antisense oligonucleotides

Because of the lack of potent CRF_2 receptor antagonists until recently, studies of the role of these receptors in behavioural responses were addressed using antisense oligonucleotide administration. This technique has been used to downregulate the expression of CRF_1 and CRF_2 receptors. Several reports demonstrate that intracerebroventricular (icv) administration of antisense oligonucleotides to CRF_1 receptors have anxiolytic effects. These effects are associated with decreased CRF binding in the hypothalamus and cortex (Schöbitz et al., 1997). Infusion of CRF_1, but not CRF_2 receptor antisense oligonucleotides has an anxiolytic effect in the elevated plus-maze after social defeat (Liebsch et al., 1999). Taken together, studies with the antisense oligonucleotides to CRF_1 receptors confirm the findings with selective CRF_1 antagonists, that anxiety-like behaviors are mediated by CRF_1 receptors.

The functional significance of CRF_2 receptors remains unclear. It has been suggested that CRF_2 receptors play a role in anxiety-like behaviours, since the administration of urocortin, (which has a higher affinity for CRF_2 receptors than CRF) produced anxiogenic effects in mice (Moreau et al., 1997). However, downregulation of CRF_2 receptors did not affect anxiety-like behaviours (Heinrichs et al., 1992; Liebsch et al., 1999). Furthermore, there were no

effects on general locomotor activity in an open field or on the spatial learning in a Morris water-maze (Liebsch et al., 1999). However, CRF_2 receptor antisense oligonucleotide treatment selectively affected performance of rats in the forced swim test without influencing other behaviours, suggesting a role for CRF_2 receptors in coping behaviour in stressful situations (Liebsch et al., 1999). Smagin et al. (1998a) studied feeding responses and demonstrated that the suppression of food intake induced by either icv injections of CRF or urocortin can be significantly attenuated by icv administration of antisense oligonucleotides to CRF_2, but not by administration of the selective CRF_1 antagonist, NBI27914.

4. Receptor-deficient mice

Generation of CRF_1-receptor-deficient mice has been reported by two groups (Smith et al., 1998; Timpl et al., 1998). Timpl et al. generated a mouse with a truncated protein instead of functional CRF_1 receptor, unable to activate adenylyl cyclase. Smith et al. replaced the last 12 amino acids of the first extracellular domain, which resulted in a non-functional CRF_1 receptor protein. Both groups obtained similar results in behavioural tests. When tested in the light–dark box, mice lacking CRF_1 receptors showed less anxiety-related behaviour. In the elevated plus-maze, CRF_1 receptor-deficient mice visited and spent more time in the open arms of the apparatus, indicating a reduced anxiety response (Smith et al., 1998). In another test, mice were subjected to a forced alcohol-drinking procedure and tested under withdrawal conditions in the light–dark box. During withdrawal, CRF_1 receptor-deficient mice showed a lower latency to enter the lighted compartment that wild type mice made more entries and spent more time within the lighted compartment (Timpl et al., 1998).

5. Conclusions and perspectives

The evidence provided above is insufficient to fully understand the role of CRF receptor subtypes in the behavioural responses in stress. The lack of pharmacological tools, such as specific CRF_2 agonists and a limited number of CRF_2 antagonists, as well as specific CRF_1 agonists have hindered studies of the functions of the receptor subtypes. Studies of CRF-deficient mice have shown that mice lacking CRF express minimal impairments in their behaviour and in responding to stressors and administration of CRF or urocortin (Dunn and Swiergiel, 1999; Swiergiel and Dunn, 1999; Weninger et al., 1999). Moreover, the expression of urocortin is not changed in CRF knockout mice, suggesting that urocortin does not usurp the role of CRF in the behavioural responses (Weninger et al., 1999). It has been proposed that another peptide or peptides can act through CRF receptors to elicit behavioural responses in the absence of CRF (Weninger et al., 1999; Dunn and Swiergiel, 1999). Steckler and Holsboer (1999) have suggested that CRF_1 receptors may be more concerned with cognitive aspects of behaviour, including attention, executive functions, emotions, and possibly, learning and memory, while CRF_2 receptors primarily influence processes necessary for survival, including feeding, reproduction and defense.

Development of new potential CRF receptor agonists/antagonist, as well as studies of CRF_2 receptor deficient mice and the search for new peptides from the CRF family will help us to understand the role of CRF in stress-related behavioural processes, and in the development of new therapeutic approaches to treat stress-related diseases.

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